BACTERIAL DISEASE OF *PISUM SATIVUM*

BY

DOROTHY M. CAYLEY

IMPERIAL BUREAU OF MYCOLOGY
26 JUN 1921

FROM THE JOURNAL OF AGRICULTURAL SCIENCE, VOL. VIII. PART IV, DECEMBER 1917

CAMBRIDGE
AT THE UNIVERSITY PRESS

[*Reprinted from the Journal of Agricultural Science*, Vol. VIII. Part IV.]
[*All Rights reserved.*]

BACTERIAL DISEASE OF *PISUM SATIVUM*.

By DOROTHY M. CAYLEY

Minor Student, John Innes Horticultural Institution, Merton, Surrey.

A short preliminary note on this disease was published Nov. 1912 (4), and since that date further investigations have been carried out into the life-history of the organism, and its effect on the host plant. As I there showed the disease is caused by a bacterium for which I propose the name *Pseudomonas seminum*.

As stated in the above mentioned preliminary note, the disease in this district, at all events, is a serious one, and now further details have come to hand showing that it occurs in other parts of the south of England. The disease causes considerable damage at this Institution where peas are grown fairly extensively for experimental purposes.

In the case of peas grown thickly in rows for culinary purposes, the diseased plants, unless they occur in large numbers, are either not noticed, or ignored, or their condition is attributed to one or more of the many diseases or insect pests to which eating peas are liable.

The most remarkable characteristic of this disease is that the organism is actually present in large numbers in the tissues of the cotyledon, and sometimes in the young embryo itself. Samples of diseased peas from Sleaford in Lincolnshire, sent to the Board of Agriculture in 1915 and forwarded to me for examination, proved to be infected with *Ps. seminum*, and showed the typical discoloration in the centre of the cotyledons. Other samples from various retail seedsmen have been examined from time to time, some of which have been absolutely healthy, others slightly diseased. The conclusion arrived at from the examination of these samples is that the disease is becoming more generally distributed. Whereas in 1913 and 1914 it was comparatively easy to find samples without disease, this year, 1916, considerable difficulty was experienced in procuring sterile seeds for experimental purposes, free from blemish inside or on the seed-coat.

Besides bacteria, *Sclerotinia* is very frequently present in the seed-coat, even after seed-sterilisation for 15 to 20 minutes in 1 per cent. corrosive sublimate. This treatment does not prevent germination in normal healthy seeds, but seeds attacked by *Sclerotinia* in many cases fail to germinate at all, or if they do germinate only make weak growth.

Such samples grown for culinary purposes under ordinary conditions in the open would give a good average crop, but so little is as yet known as to the external conditions controlling this disease, that a bad outbreak in any one year is not beyond the limits of possibility.

The disease occurs on both heavy and light soils. The earlier tall varieties of peas are much freer from disease than the later more succulent varieties. External temperature together with succulent growth greatly influences the development and spread of the organism in the plant.

The disease was especially bad during the first two or three summers after this Institution was opened, namely, 1910, 1911, and 1912. The land, which had previously been used as a market garden, is a heavy clay with an impervious subsoil of blue London clay. When the land was first taken over the drainage was quite inadequate and parts were liable to be under water in wet weather in the winter. Latterly, as the tilth has been improved by drainage, etc., the effects of the disease have not been so marked. In the case of a light soil, the previous history of which was known, the disease occurred on freshly broken poor grass-land, which had not carried a crop of peas for many years, if ever. This soil was somewhat deficient in lime.

In order to detect the presence of bacteria in the tissues, the young fresh succulent growth should be examined, where large numbers of the organism can be seen in the motile stage, in the cells of the cortical tissue near the phloem. As far as can be judged the organism penetrates and travels up through the plant in the motile stage, but it only remains motile if there is a considerable amount of moisture present.

Several instances of seeds with foreign organisms in their interior, both symbiotic and parasitic, have been recorded of recent years. The only strictly analogous case to this pea disease, in which however the parasite is a fungus instead of a bacterium, is that of Barley Smut (*Ustilago hordei nuda*), described by Broili and Schikorra in 1913(3). These authors found the mycelium of the Smut both in the endosperm and the embryo of the resting seed. Another interesting point of similarity is that the fungus, during the earlier stages of the growth of the host plant, grows up with it in a state of semi-symbiosis without causing any very marked disturbance until the flower spike begins to develop.

On the other hand, in the case of the Rusts in the Gramineae, although Beauverie(2) observed sori of uredospores, teleutospores, and mycelium in the parenchymatous tissue of the caryopsis near the furrow, and in the pericarp beyond the region of the furrow, the mycelium could not penetrate the layer of strongly cuticularised tissue immediately outside the aleurone layer of the seed itself, unless this protective layer had been ruptured from some cause or other, in which case, bacteria were generally found to be present.

The most marked instance of a symbiotic bacterium inside the seed is that of *Ardisia crispa* and *A. crispa* var. *compacta* (Miehe(11)). The bacteria occur in the plant in nodules or pockets at regular intervals 30—50 mm. apart at a short distance from the edge of the leaf, in the buds and inside the seed between the embryo and the endosperm. Miehe considers this a case of strict symbiosis.

Instances of seed-coat parasitic infection and infection of the ovarial tissues are not uncommon. In the mycorhiza-bearing plants the mycelium of the symbiotic fungus in the Ericaceae was also found to be present in the seed-coat, but not in the internal tissues of the seed (Rayner(13)).

SYMPTOMS OF THE DISEASE.

The general symptoms are as follows: in mild cases, after germination, the shoot can develop normally, but in bad cases it is frequently abortive, brown, and dead at the tip, and laterals grow out prematurely to take the place of the main shoot (Plate V, fig. 1). Quite early in the development of the plant, when the plumule is from half an inch and upwards in length, light brown longitudinal streaks can sometimes be seen on the stem and root. In very bad cases little or no germination takes place. After this stage no further definite signs are noticeable till about the flowering period. Then the development of the disease depends a good deal on the external conditions. If the weather is warm and dry, and the plants are growing vigorously, the disease develops rapidly, and in a few days the plants may become unhealthy and change colour. The stem turns slightly brown and looks somewhat water-soaked. Brown longitudinal streaks appear at the base of the petioles on either side of the stem which is continuous with the midrib of the leaf. The streaks sometimes split open and dry out. The collar may be badly disorganised. The leaves become spotted and yellowish in colour, with darkened veins. Sometimes the surface of the pod is roughened or embossed. This is caused by the enlargement of cortical

cells, in proximity to other cortical cells filled with bacteria. If the disease is developing rapidly the younger portions of the plant show discoloration and fail to develop properly. Except in bad cases the plants can grow to full height, can flower and set a fair crop of seed, but on examination the cotyledons of the seed show the typical brown discoloured area in the centre of each cotyledon, which may be limited to a mere spot, or, on the other hand, nearly the whole cotyledon may be involved. The young embryo, especially the plumule, can also become infected. In bad cases there is frequently a cavity in the centre of each cotyledon. Sections of young green diseased cotyledons show large numbers of bacilli in various stages of development, both in the cells and in the intercellular spaces. If one pea is diseased all the other peas in the same pod are diseased to an equal extent. Healthy and diseased seeds have never been found in one and the same pod.

A large number of plants have been examined and the pods and seeds recorded in the order of development from below upwards, and it is a general rule that the first set pods show less discoloration of the internal tissues of the cotyledons than those developed later. Frequently the first set pod is free from infection. I venture to suggest in explanation of this, that the external temperature was too low for the rapid development of the organism, and the pods were able to mature sufficiently to resist infection before the organism could get the upper hand. The organism can only penetrate uninjured tissues when they are very young.

In some respects the symptoms resemble those of the formidable "Streak" disease of the Sweet Pea (*Lathyrus odoratus*) and in my preliminary note I suggested that Sweet Pea Streak was due to bacteria and not to *Thielavia basicola* as previously held (5,10). Early in the following year, 1913, a paper was read before the American Phytopathological Society, Cleveland, by J. J. Taubenhaus (9), and a short extract published in the *Gardeners' Chronicle*, showing that Streak in Sweet Peas was a bacterial disease due to *Bacillus lathyri*. This bacillus was found by the authors to occur on a wide range of other host plants, including Forage Lathyrus, Red Clover, Alsike Clover, Soy Bean, Wax Bean, Lima Bean, and Cowpea.

I have been unable to obtain further details than those published in this abstract, as to the biochemical characteristics of the organism, but a yellow organism isolated from a Sweet Pea suffering from Streak, which I took to be the same organism as that obtained by the above author, proved not to be *Ps. seminum*. Cross inoculations were

not tried, as Streak of Sweet Pea and bacterial disease of eating pea are both so prevalent at this Institution that the results would be wholly unreliable.

So far *Ps. seminum* has not been found on any other host plant; there is however no doubt that the organism occurs in the soil, as healthy peas sown on infected land can develop the disease. Judging from the fact that the organism can live a long time in artificial liquid media, it can probably live for years in the soil. To give one example of many records illustrating this capacity, a tube of sterile soil-extract was infected with a pure culture of *Ps. seminum*, and it grew well when plated after eight months and two days. Old platings from other nutrient media gave similar results.

INFECTION OF THE HOST PLANT.

As mentioned above, the organism can only penetrate uninjured tissues when they are very young. Plate VI, fig. 1 shows the penetration of the epidermal cells of the young embryo of a pea germinated in sterile sand in a petri dish. It gains access into the tissues in the rod stage chiefly through the radial walls of the epidermal layer, but can also penetrate the young cuticle. The cell walls become swollen and disorganised (Plate VI, figs. 1, 2, S.W.) and the organism forces its way into the intercellular spaces and from there into the cells themselves. The nucleus is often attacked, the cytoplasm destroyed, and the cells dry out and rents occur in the tissues. These rents are particularly marked in dry weather and occur chiefly at the nodes, immediately below the base of the petiole of the leaf. Plate VI, fig. 2 shows the cells of the phloem parenchyma of a young stem, in which two cell walls have been broken down; and Plate VI, fig. 3 a cell of the cotyledon of a badly diseased pea, after germination. This last figure shows very clearly the penetration of the bacilli into the cells.

The organism occurs in the phloem, cambium, medullary rays, and occasionally in the pith, also in the parenchyma and vascular bundles of pods, in the tissues of the funicle, the cotyledons, and the leaves. It has no diastatic action on the pea starch. In Plate VI, fig. 3 a few starch grains can be seen intact, but most have been dissolved out by the chromacetic fixative. Unless some starch-dissolving fixative is used it is not possible to cut good microtome sections of the cotyledon. The bacilli have never been observed in the vessels. The occasional wilting of diseased plants is probably due to some other cause, such as

Fusarium or injury by soil insects. How the organism passes into the interior of the seed is not known. The bacilli have never actually been seen passing up the micropyle, but they occur in the thin-walled layer of cells lining the pod, immediately inside the sclerenchymatous layer of supportive tissue. The infection of the young seed probably occurs earlier, as all the seeds in a pod are diseased to an equal extent. It is more probable that infection takes place through the parenchyma and phloem of the vascular bundle which passes up the funicle, and which is connected with the vascular strands running along the midrib of the pod. Cultures of the organism, however, have been obtained from the cells of the thin-walled innermost lining of the pod, which cells, under moist conditions, sometimes grow out into unicellular hairs.

The interior of a very young pea is filled with sap, and it was thought that the organism might be present in this sap, but smears made of this sap and stained with bacterial stains failed to reveal the organism in the rod stage.

Morphology of the Organism.

Ps. seminum is a large motile rod, with a single polar flagellum, attached rather to one side of the pole. The length of the rod varies considerably according to the age of the colony, and the medium used. On solid media, taken from a young colony the length is from 4μ to $5\mu \times 1\mu$, but in liquid media the rods can attain a length of from 9μ to 10μ before division. Chains of four to five rods reaching in length from 30μ to 40μ are not infrequently met with in young broth cultures. On solid media the organism soon passes into the beaded state. This consists of oval bodies, either separate or occurring in chains. They are formed inside the rods and are approximately as broad as the rods. They are highly refractive when alive, and might easily be mistaken for spores. When stained with ordinary bacterial stains such as Gram, Gentian Violet, Carbol Fuchsin, Methylene Blue, etc., these oval bodies take the stains very lightly. The margin is stained rather more than the interior, which remains almost colourless. As repeated efforts to stain them with spore stains failed, it was thought that the cultures were impure, and that there were two organisms present. In order to separate the two organisms numbers of sub-cultures were made in various liquid media including sterile water only, and then replated on to solid media, but without success. The oval and rod stages occurred persistently. Moreover, the colonies varied considerably in size and shape, all, however, giving the same characteristic reaction to litmus

milk. It was not until the development of the living oval bodies into the long rod stage was watched under the microscope that it could be definitely proved that both forms were stages of the same organism (see Plate VII, figs. 1—10).

A thin film of acid pea agar agar (1 per cent. normal HCl) was obtained by infecting the medium with a pure culture of *Ps. seminum*, and then before the medium had time to solidify, a sterile coverslip was dipped into it, so that only one side touched the medium. The coverslip was then placed on a sterilised moist-chamber sunk slide, and fixed in position by two or three drops of paraffin wax. The slide was then placed upon a warm stage and kept under observation for twelve hours. Owing to the layer of air between the coverslip and the slide the dark ground illumination substage could not be used. However, by careful adjustment, it was possible to get a satisfactory lighting. Unfortunately, after twelve hours the coverslip was cracked while focusing, so that details of the beading stage, which, it will be noticed is just beginning in *f* group, Plate VII, fig. 10, could not be followed out. In a hanging drop the organism tends to become more and more difficult to see the older it grows. One rather interesting point to be noticed in these figures is that, when division is completed, the full-grown daughter rods come to lie parallel to one another instead of end to end, Plate VII, figs. 4—10.

This suggests that the daughter rods grow in length in the region somewhere near the point of division, especially as, in this case, the organism is growing on a solid agar medium, in which entire change of position would not be easy. The bending of the rod before complete division also rather supports this suggestion. In one case, *e*, it will be observed that complete separation has not occurred until the third division, and the rods lie end to end. It is only in fig. 8, that the marked bending appears in this group. Group *e* is not figured in figures 9 and 10 as it grew too large for the whole of it to appear within the field of the microscope, and also became overgrown by a neighbouring group outside the field. I venture to suggest that these observations, together with other factors, may account for the puzzling variability of the macroscopic appearance of the colonies. There were slight indications from time to time of the formation of thin capsules surrounding the rods, but with regard to this the lighting did not allow of any very accurate observations. These capsules, however, have been observed in stained sections. Smears of old cultures on solid media, stained for spores, do not give any very satisfactory results, as the organism gets massed together

without any very clear definition. The best spore-staining results were obtained from pellicles in liquid media and from growths on potato. The spores can be seen, Plate VII, fig. 14, in the middle or to one side of the oval bodies, so that they appear to protrude. The surrounding substance of the oval bodies seems to swell gradually and become disintegrated so that the spores eventually lie embedded in a pseudo-zooglaeal mass.

I consider the oval bodies to be a kind of involution form as they sometimes vary in size and shape. The germination of spores has not been observed. It is difficult to identify the organism in microtome sections of the older parts of the plants, or in the dry cotyledons, owing to the oval bodies and the pseudo-zooglaeal masses. They can hardly be distinguished from the surrounding heavily stained disorganised tissues of the host plant. In the case of dried peas it is only after they have been kept under moist sterile conditions conducive to germination from 10—14 days, or even longer, that the rod stage can be definitely seen. In hand sections of the young succulent parts of the plants, the rods can be readily seen moving about in the cortical cells, although the tissues appear healthy and turgid. The organism can be isolated with ease from the centre of the cotyledons and from the diseased stems. If sufficient care is taken, almost pure cultures can be obtained by infecting tubes of nutrient broth with a small portion of the tissues of the stem or cotyledons, although in the case of cultures from the dry cotyledons the culture tubes have to be kept in the incubator at least 10 days before a satisfactory plating can be obtained. The organism grows well at a temperature of 25° C.

Variability in General Characteristics of Colony.

Differences in macroscopic appearance in cultures on artificial media do not necessarily mean impurities. Capsular organisms, or those which produce slime, may develop, and mostly do develop, giant colonies(1). Individuals get stuck together, but on the other hand there is always the danger of foreign organisms being caught up in the slime which may prove to be very difficult to separate out. Marshall Ward(14) in 1895 maintained that variation in form, rate of growth, and other characteristics of plate colonies result from much slighter variations in the medium and other environmental conditions than is generally recognised: also, that organisms are affected by the vicissitudes to which they have been subjected previous to culture. First platings of the wine bacterium as observed by A. J. Perold(12)

gave colonies of different appearance, but later platings showed uniformly pure cultures. Among others *Bacillus mycoides* (6), *Bacillus megatherium* (7), are instances of organisms non-pathogenic to man, which show considerable colony variability.

Ps. seminum also shows considerable variability in size and general characteristics of its colonies on artificial media. The fact that the organism produces a thin capsule round the rods when they come to rest after the motile stage, and has its spores embedded in a kind of matrix, may partly account for the variability shown. In liquid media it hangs together in long chains, and as I have already pointed out, this phenomenon of incomplete division can also occur on solid media, and may be a possible explanation of the occurrence of straggling outgrowths in the submerged, and lobing or radiations in the surface colonies.

The two forms of colonies most frequently met with in platings of *Ps. seminum* are (1) surface opaque white or whitey-buff, more or less circular, and (2) surface less opaque, more or less widely radiating colonies, with their corresponding lens-shaped and straggling submerged colonies (Plate VII, fig. 13). Repeated tests as to their reaction to litmus milk showed identical results with both colonies. They also give the same reaction with sugars. There is no doubt that the amount of water in the medium greatly influences the size and length of the lobes or radiations in the spreading colonies. Treatment of the organism previous to plating may also have some effect. If the bacillus is isolated from the young green cotyledon or the growing stem, it tends to throw spreading colonies; whereas more or less circular surface colonies result from young cultures from dry cotyledons incubated in peptone beef broths. If pure cultures of first platings are kept for some time in liquid media, so that a pellicle or flocculent sediment is formed, the resulting colonies then vary considerably from the original colony.

To give illustrations of this, a tube of alkaline (1 per cent. normal NaOH) peptone beef broth was infected with a surface opaque whitish circular colony from a dry cotyledon, and, replated after five months and twelve days on acid (1 per cent. normal HCl) pea agar agar, gave a straggling surface colony. On the other hand a tube of sterile soil extract was infected with a straggling colony and replated on neutral pea agar agar, and gave the following:

(1) More or less circular opaque colonies, inclined to be lobed.

(2) One large semi-transparent spreading colony which almost completely covered the whole surface of the petri dish.

(3) Radiating semi-opaque colonies.

(4) Submerged colonies, flat disks with roundish lenses in the periphery (Plate VII, fig. 12).

This plate to all external appearances looked impure, but careful tests of all these four types of colonies gave identical and typical reactions with litmus milk. A good deal also depends upon the morphological stage of the organism at the time of plating. Replatings from one solid medium to another tend to retain their original colony characteristics, but ringing the changes between solid and liquid media only led to further puzzling results and occasional impurities.

The inference to be drawn from these facts is that there are possibly two strains of *Ps. seminum* which differ only in their shape of colony. One strain may be more capable of complete division than the other. The proportion of definite rods to oval bodies is greater in the radiating colonies than in the circular surface colonies.

Platings from young cultures and from very old cultures in liquid nutrient media, give much more uniform results than platings from the intermediate stages. Also platings of the pellicle are naturally much more varied than when the pellicle is avoided and platings made from the broth itself. The colonies also vary in general characteristics according whether the medium is acid, alkaline, or neutral (Plate V, figs. 3, 4, 5) although the organism grows well on all three. Plate V, figs. 3 and 4 show magnifications of giant colonies of the same culture on neutral and alkaline media, and it will be seen that on neutral pea agar agar the individual submerged colonies are much more definitely lens-shaped than on the alkaline medium.

Plate V, fig. 5 also shows the different growths although the platings are rather thick, and Plate VII, fig. 12, drawings of varying shapes in submerged colonies, all of which give the typical litmus milk reactions.

Biochemical Reactions.

Ps. seminum is a facultative anaerobe and will grow to the bottom of a closed agar stab.

Beef broth. The organism grows well on acid (1 per cent. normal HCl), neutral and alkaline (1 per cent. normal NaOH) broth. A pellicle is formed, which, on shaking, sinks to the bottom of the liquid and forms a stringy precipitate. This precipitate gradually disappears and the liquid becomes turbid. Both oval bodies and rods strung together in long chains occur in the pellicle, but if the latter is avoided, rods and

no oval bodies show in smears made of the liquid itself. Most pellicle occurs on alkaline beef broth.

Potato. The organism grows well on sterile potato chunks. The growth forms a layer of creamy consistency over the surface, and becomes contoured or creased when the potato begins to dry out. There is no discoloration, but occasionally a pinkish tinge appears in the bacterial growth both on potato and on the testa of the pea seed. Plate V, fig. 2 shows a two-year old pea enlarged, which was sterilised in 1 per cent. corrosive sublimate for 15 minutes, having been previously moistened in sterile water under reduced pressure. It was then placed in sterile sand to germinate. There was, however, no germination. The seed was dead, but a prolific contoured bacterial growth developed on the testa, slightly pinkish in tinge, which proved to be a pure growth of *Ps. seminum*. Microtome sections of the testa covered with bacterial growth showed masses of long rods and oval bodies embedded in a matrix, composed partly of disorganising rods.

Gelatine. Gelatine is rapidly liquefied. The liquefaction in a stab is elongated napiform at first, but finally the whole of the gelatine in the tube is liquefied. A pellicle and precipitate are formed as in the peptone beef broths. There is no odour with any of the artificial media.

Sugar reactions. Stabs made in litmus gelatine with 1 per cent. dextrose, saccharose, and lactose respectively show liquefaction and acid formation with dextrose and saccharose, but only liquefaction and no acid with lactose. The development with lactose is slower than with the other two above-mentioned sugars. No gas is formed with any of the sugars.

Litmus milk. The reaction with litmus milk is very characteristic. The litmus is slowly reduced without the formation of acid, and passes gradually through a greyish tinge to a clear apricot colour. The shade of apricot varies slightly according to whether the original litmus is pale and alkaline, or darker and rather on the acid side. In the latter case the apricot colour is rather darker. There is no coagulation, and the milk gradually becomes quite clear, with the usual bacterial sediment. This apricot reaction is very typical of *Ps. seminum*. Both spreading and circular colonies give exactly the same reaction. If the tube is shaken when the clear apricot colour has formed, the liquid turns pinkish in colour in a few minutes, but the clear apricot colour returns after a few hours. This pinkish colour may also appear if the tubes are taken out of the incubator and cooled down to about 14° C., but the apricot coloration returns when the cultures are replaced in

the incubator. This pinkish colour cannot be due to acid, as all the blue coloration of the reduced litmus has disappeared. The colour then changes from apricot to deep sherry, and finally the liquid is a clear, deep wine shade with a heavy bacterial sediment. The action on litmus milk is slow.

Glycerine. There is good growth on glycerine without the formation of gas or acid.

Nitrate reduction. Nitrate is reduced to nitrite without the formation of gas. The organism soon loses its power of reducing nitrates when grown on artificial media. Only young cultures from the growing stem and freshly incubated cultures from the dry cotyledon should be used for this test. The organism however retains its power of reducing litmus a very much longer time. The apricot reaction occurs with cultures some months old. The nitrite test used was that recommended by Jordan(8), namely:

After four days incubation in nitrate broth (0·1 per cent. peptone, 0·02 per cent. nitrite-free potassium nitrate) add to 3 c.c. of the culture 2 c.c. of each of the following:

(1) Sulphanilic acid solution made by dissolving 8 grammes of the purest sulphanilic acid to 1000 c.c. of 5N acetic acid (sp. gr. 1·041).

(2) α-Amidonaphthalene acetate solution prepared by dissolving 5 grammes solid α-Naphthylamine in 1000 c.c. of 5N acetic acid and filtering through absorbent cotton wool.

The development of a rose colour indicates the presence of nitrites.

Diastatic action. The organism has no diastatic action on pea starch.

Stab Cultures. The line of puncture is indefinitely beaded and growth occurs down to the bottom of a closed agar stab.

Reactions to stains. In the rod stage *Ps. seminum* takes the usual bacterial stains very readily. It is gram-positive and non-acid-fast. But as already stated above the oval bodies only stain lightly. The flagellae are also very difficult to stain; the best results were obtained with van Ermengen's flagella stain.

Isolation of the organism. The organism has been isolated from the centre of both green and dry cotyledons, from the stem, from the pod, and from the hair-like outgrowths sometimes found lining the pod.

Motility. When the parent rod is about to divide, it has a slow sinuous motion, but when division is completed, the daughter rods have been seen at first to revolve very rapidly on their own axes, as indicated by the arrows in Plate VII, fig. 14 *a*, and then to dart about the field of the microscope. When observed alive in the cells of the

host plant the general movement is slow, with an occasional swiftly moving rod.

Group number. The group number of this organism, according to the Description Chart of the Society of American Bacteriologists is:

Ps. 121·2323033.

So far as is known to the author, this organism has not been previously described, and has therefore been named *Pseudomonas seminum.*

INOCULATION EXPERIMENTS.

Inoculation experiments were carried out in the open, but little stress can be laid upon the results, as the disease was so prevalent throughout the experimental plots. Pea plants grown in boxes in soil partially sterilized by heat and inoculated just above the ground when the plants were one foot high, showed no disease, whereas in the open seven out of ten inoculations in the stem below the youngest leaf were successful. These contradictory results were probably due to the part of the plant inoculated. The stems of the plants in the boxes had probably matured sufficiently to enable them to withstand the spread of the organism.

Other outdoor experiments were tried in which the young unfolded leaves were pressed apart near the growing point, the young stem pricked with a sterile needle, a drop of sterile water containing a pure culture of the organism placed on the wounded stem, and the young leaves replaced over the drop. These inoculations also gave definite results in so far as the growth was partially arrested, the leaves turned yellowish, and the upper part of the plants showed the general symptoms of the disease. However, in this case also, some of the control plants treated in the same way with sterile water only, developed the disease. Further attempts were made to grow pea plants under sterile conditions in sand in carboys and watered with nutrient salt solutions, but unfortunately the cultures did not remain sterile owing to the necessity of opening the carboys for manipulation. The seeds for these experiments were sterilized with 1 per cent. corrosive sublimate for twenty minutes, washed twice in sterile water and then placed singly in sterile petri dishes to germinate. After germination the testa was removed, the cotyledons of the seeds were forced apart with sterile instruments to see if the internal tissues were healthy, and those showing any blemishes on any part of the seed were rejected. When the first leaves had unfolded, the peas were planted in the

carboys, which were kept lying on one side throughout the experiment, great care being taken to keep them as sterile as possible during manipulation. Five days after the plants had been placed in the carboys they were inoculated with a pure culture, in sterilized water, of a surface more or less circular opaque whitish colony isolated from the centre of a diseased cotyledon. A drop of the culture was placed on a young leaf. The leaf turned browny yellow in three days, at the end of ten days the growing point of the stem was practically dead, and laterals had begun to develop. Identical results were obtained from a radiating colony, see Plate VII, fig. 13, from the same original culture, whereas the controls, treated with sterile water only, remained healthy throughout this period. Another carboy was inoculated with a mixture of the two above-mentioned colonies with exactly the same results. The main shoot died back in 10—11 days. These results are only conclusive in so far as they show that young tissues can be infected and die back when inoculated on the surface with a pure culture of *Ps. seminum*.

Pea plants grown under sterile conditions make poor growth, even when supplied with a complete nutritive solution and, as stated above, succulent growth is necessary for the full development and rapid spread of the organism in the plant. It is also difficult to obtain seed from pea plants grown under moist sterile conditions. Nearly all the flowers in the above experiments shrivelled and dropped off.

Susceptibility.

All attempts to find an immune variety of pea have been without success. A number of different varieties of mid-season and late culinary peas were grown to test their resistance to the disease, but all varieties, so far, have been found to be more or less susceptible. The variety which proved to be most resistant was Sutton's Improved Petit Pois, which, sown on infected land, gave from 49 to 50 per cent. healthy plants. Seeds saved from these selected healthy plants and sown again on the same plot the following year also gave approximately 50 per cent. healthy plants.

Duke of Albany and Ne Plus Ultra are very susceptible, the average number of healthy plants of Duke of Albany being 14 to 15 per cent. The growing of the Ne Plus Ultra for Mendelian experiments at this Institution had to be discontinued owing to its extreme susceptibility.

As mentioned above, the taller varieties of early peas show considerably less disease than the later varieties, partly, it is believed, because of their less succulent growth and earlier development, which

latter enables the plant to attain sufficient maturity before external conditions are conducive to the rapid development of the organism.

Thanks to the courtesy of Mr F. J. Chittenden, I was able to examine a large number of different varieties of early peas grown at the Wisley Early Pea trials in 1915. The plants had all but ripened off, and only one variety was found which showed the typical discoloration of the cotyledons, although, judging from external appearances there were many doubtful cases. A row of Duke of Albany, growing in an adjacent plot but in a much earlier stage of development, showed the typical discoloured patch in the centre of the cotyledons.

It must, however, be pointed out that the disease is not so prevalent at Wisley as it was at this Institution, and that the trials, so far as the disease is concerned, cannot be taken as giving conclusive results. But general observations on pea plants grown on soil known to be infected illustrate this point much more conclusively. There is also the possibility that, in the two cases in point, of diseased varieties occurring at Wisley the disease may have been introduced with the seed, as it is impossible to tell from the external appearance whether a seed is healthy or diseased. It can thus be seen that it is very difficult to get results which are really reliable.

Of the dwarf succulent early varieties Chelsea Gem and Little Marvel, and of the later varieties Ne Plus Ultra and Duke of Albany, are particularly susceptible. Sugar peas and rogue peas can also become badly diseased when grown on infected land.

It is hoped, after further trials, that it may yet be possible to find an immune variety, so that by breeding this immunity may be transferred to some of the excellent varieties of culinary peas already on the market.

REMEDIES.

So far no cure is known: in fact, a cure is hardly possible in this case of a succulent annual, especially when the seed itself may be infected with the parasitic organism. The precautions to be recommended are the rotation of crops, early sowing, and general healthy cultural conditions as to drainage, sufficiency of lime, etc., and the rejection, where possible, of seeds known to have come from diseased plants. The haulms from an infected crop, together with the sticks, should be burnt as soon as possible after the crop has been gathered. Partial sterilisation of the soil is useless, as *Ps. seminum* is a sporing organism; it is also impracticable owing to the amount of labour required

and the expense. Care should be taken to thoroughly clean all implements used on infected land so as not to spread infection.

Summary.

Ps. seminum is a motile sporing facultatively anaerobic bacillus capable of growth on acid, neutral and alkaline media.

The chief characteristics of the disease caused by this organism is that it is present in the interior of the seed, forming a discoloured area in the centre of each cotyledon. It is not possible to tell from the exterior of the seed whether it is, or is not, infected.

External infection takes place through the young tissues, and, as far as is known, the organism is unable to spread through matured tissues.

Infection occurs considerably in advance of discoloration in the tissues.

The organism occurs throughout the plant, but has never been observed in the vessels.

It has been isolated from the interior of the seed, both dry and green, the stem, the embossed pod and the layer of thin-celled tissue which forms the innermost lining of the pod.

The organism has never been seen passing through the micropyle of the seed, although on one occasion rods were observed lying in the spaces between the cotyledons and the young embryo.

The organism passes up the plant in the motile rod stage.

The macroscopic appearance of the colonies on artificial media is very variable.

The organism develops rapidly at a temperature of 25° C. but development is slow below 20° C.

The motile stage only lasts a short time unless there is a considerable amount of moisture present. This is followed by the beaded stage, and finally spores are developed inside the beaded stage, and the spores eventually lie embedded in a pseudo-zooglaeal matrix.

All varieties of culinary peas so far examined are more or less susceptible.

Germination is not arrested, but in bad cases the development of the plant is retarded owing to the dying back of the main shoot and the development of laterals to carry on the growth of the plant.

DESCRIPTION OF PLATES.

PLATE IV.

Fig. 1. Peas germinated in a cool greenhouse in soil partially sterilized by heat.
1, 1, 2, 2. Diseased seed.
5. Control, healthy seed.

Fig. 2. A. Healthy plant, just before flowering period.
B. Four diseased plants of the same age.

PLATE V.

Fig. 1. A. Seven diseased seeds germinated in sterile sand in a petri dish.
B. Typical healthy seedling grown in a pot.

Fig. 2. Pea seed enlarged, two years old, kept in sterile sand to germinate. No germination. The contoured growth (*a*) on the testa is a pure growth of *Ps. seminum*.
(*b*) Grains of sand still adhering.

Fig. 3. Giant colonies of *Ps. seminum* enlarged, growing on neutral pea agar agar.
(*a*) Submerged colony.
(*b*) Surface colony.

Fig. 4. Same culture as in Fig. 3, but growing on alkaline pea agar agar.
(*a*) Submerged colonies.
(*b*) Surface colony.

Fig. 5. Platings of *Ps. seminum*.
(*a*) Acid (1 per cent. normal HCl) pea agar agar.
(*b*) Alkaline (1 per cent. normal NaOH) pea agar agar.
(*c*) Neutral pea agar agar.

PLATE VI.

Fig. 1. Microtome section of epidermal cells of young embryo of pea germinated in sterile sand, showing penetration of bacteria into uninjured cells and into the intercellular spaces.
S.W. Swollen cell wall.
I. Intercellular space, filled with bacteria, stained Carbol Fuchsin and Licht Grün.

Fig. 2. Microtome section of phloem parenchyma of young diseased stem infected with *Ps. seminum*.
S.W. Swollen disorganised cell wall.
B. Bacteria.
H. Healthy cell wall.

Fig. 3. Microtome section of cotyledon of pea germinated in sterile sand.
B. Bacteria.
D. Disorganised cell walls.
H. Healthy cell wall.
I. Intercellular spaces filled with bacteria.
S. Starch grains.

PLATE VII.

Growth of *Ps. seminum* during a period of twelve hours, on a film of acid (1 per cent. normal HCl) pea agar agar.

Fig. 1. Oval bodies, 12.20 p.m.

Fig. 2. 3.10 p.m. Rods developed from the oval bodies.

Fig. 3. 3.40 p.m.

Fig. 4. 3.55 p.m. Complete division occurred in groups *a* and *f*.

Fig. 5. 4.25 p.m. Second division beginning in *a* group, parallel position in *f* group. Complete division in *c* group and beginning of parallel position.

Fig. 6. 5.25 p.m. Third division in *a* group. Parallel positions in *c* group after second division. Incomplete division in *e* group. Growth beginning in *g*.

Fig. 7. 6.30 p.m. Still incomplete division in *e* group. Development in *g*. Parallel growth in *d* and *f* groups.

Fig. 8. 7.30 p.m. Complete division in *e* group for the first time, after four sub-divisions. Parallel growth in *b* group.

Fig. 9. 9.5 p.m. Division occurring in *g* group. Parallel growths in *a*, *b*, *f*, *d* groups. One very long rod in *c* group.

Fig. 10. 11.50 p.m. Complete division and beginning of parallel growth in *g* group. Beading showing in *f* group.

Fig. 11. Flagellae of *Ps. seminum*.

Fig. 12. Different types of submerged colonies of *Ps. seminum* grown on different media.
(*a*) Submerged colony on acid pea agar.
(*b*), (*c*), (*d*). Submerged colonies on alkaline pea agar.
(*e*), (*f*). Submerged colonies from green peas on acid pea agar.
(*g*) Submerged colony on neutral pea agar.

Fig. 13. (*h*) (*h*) Spreading surface colonies.
(*i*) (*i*) Round surface colonies, on acid pea agar agar, plated after three months' incubation of a piece of the centre of the cotyledon of a dry pea.

Fig. 14. Showing different stages of *Ps. seminum*. Note capsules, oval bodies of various shapes, and spores.

BIBLIOGRAPHY.

1. Benecke, W. *Bau und Leben der Bakterien*, 1912.
2. Beauverie, J. *Comptes Rendus*, Vol. clvi, May 1913; clvii, Nov. 1913.
3. Broili, J. J., and Schikorra. *Ber. d. deut. bot. Gesell.* Vol. xxxi, Part 7, 1913.
4. Cayley, D. M. *Proc. Roy. Soc.* B. Vol. lxxxvi, 1912.
5. Chittenden, F. J. *Journal Roy. Hort. Soc.* Vol. xxxvi, Part 3, 1912.
——. *The Sweet Pea Annual*, 1912.
6. Holzmüller, K. *Centralbl. für Bakt.* Abt. ii, Bd. xxiii, 1909.
7. Johnson, J. C. *Centralbl. für Bakt.* Abt. ii, Bd. xxxv, 1912.

Fig. 1.

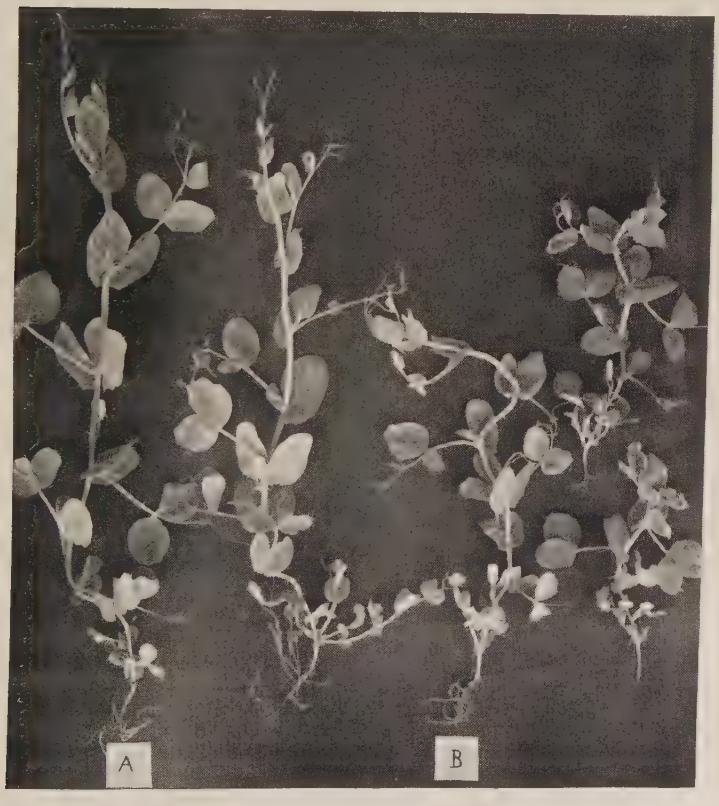

Fig. 2.

Fig. 1.

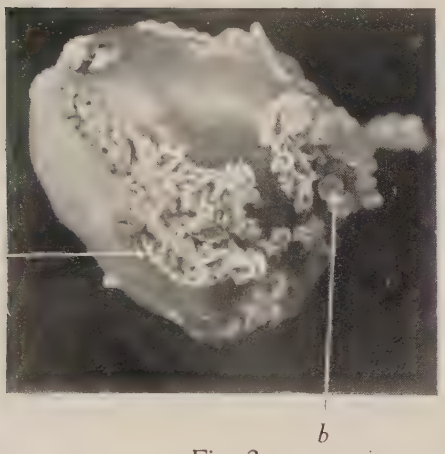

Fig. 2.

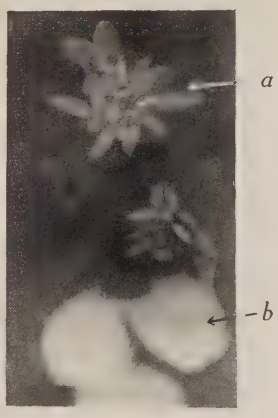

Fig. 3.

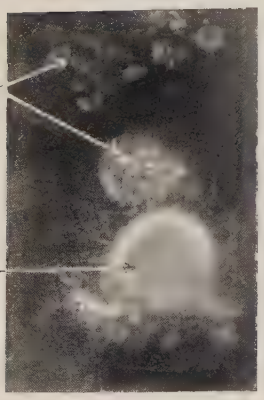

Fig. 4.

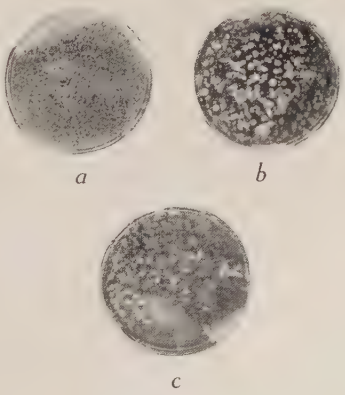

Fig. 5.

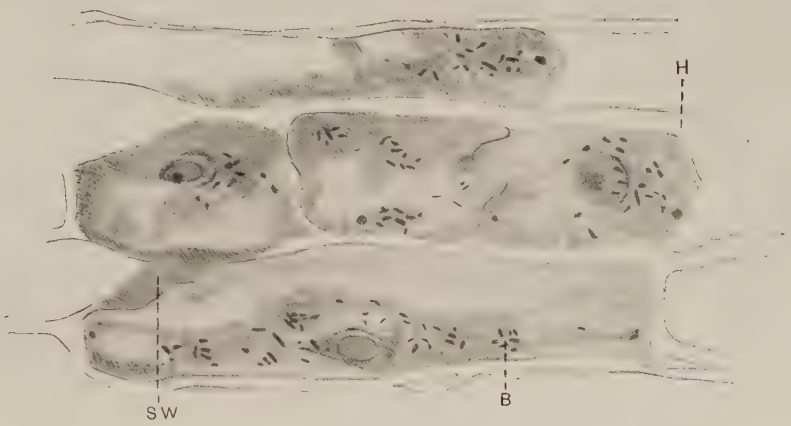

Fig. 2.

Fig. 1.

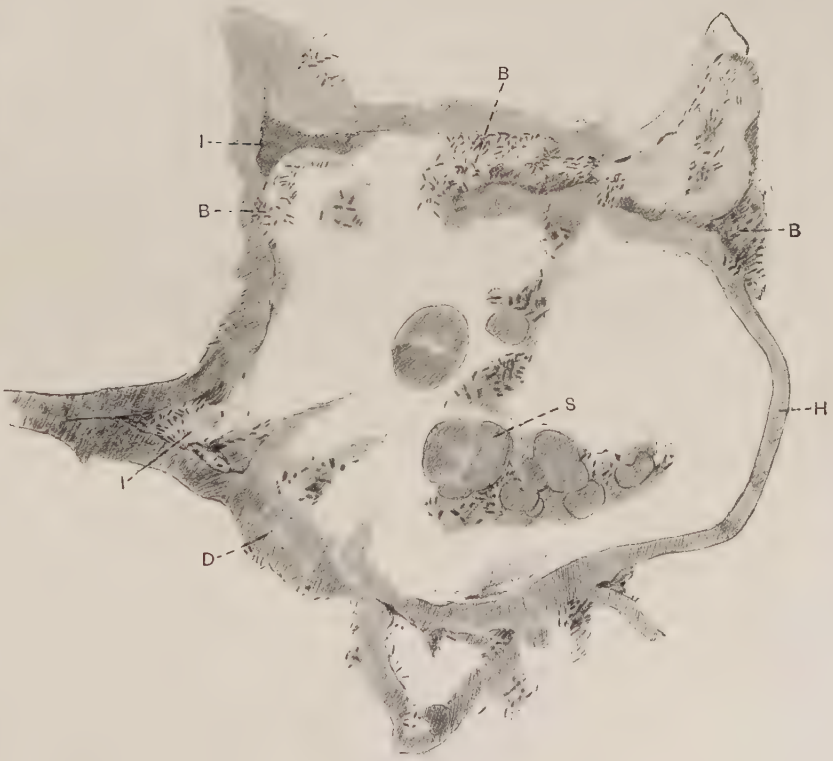

Fig. 3.

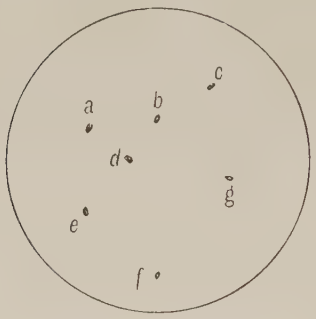

Fig. 1. 12.20 p.m.

Fig. 2. 3.10 p.m.

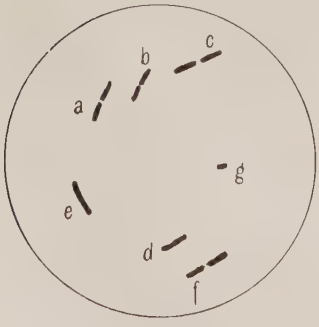

Fig. 3. 3.40 p.m.

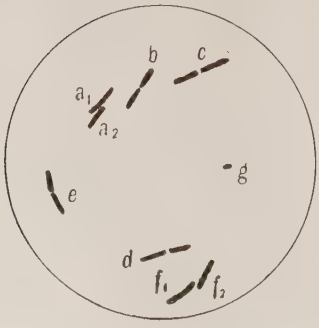

Fig. 4. 3.55 p.m.

Fig. 5. 4.25 p.m.

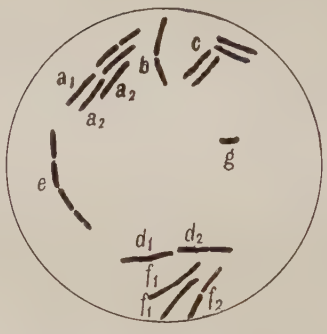

Fig. 6. 5.25 p.m.

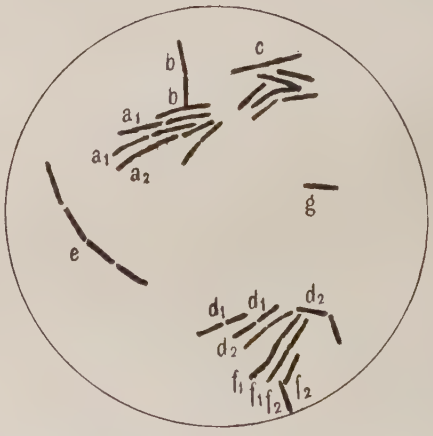

Fig. 7. 6.30 p.m.

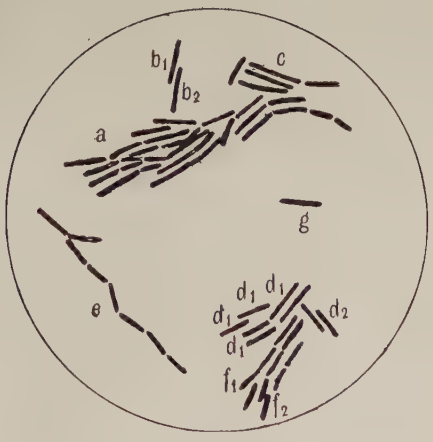

Fig. 8. 7.30 p.m.

Fig. 9. 9.5 p.m.

Fig. 10. 11.50 p.m.

Fig. 11.

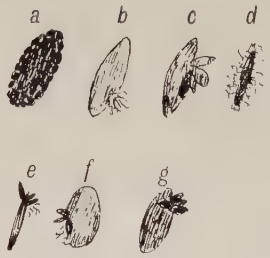

Fig. 12.

Fig. 13.

Fig. 14.

8. JORDAN, E. O. *General Bacteriology*, 1910.
9. MANNS, T. F. Taubenhaus, J. J., *Gardeners' Chronicle*, April 1913 (Abstract from a paper read before American Phytopath. Soc., Cleveland, Ohio, Jan. 3rd, 1913).
10. MASSEE, G. *Bull. of Misc. Information Roy. Bot. Gardens, Kew*, No. 1, 1912.
11. MIEHE, HUGO. *Javanische Studien, Abt. der math. phys. Kl. der königl. Sachs. Ges. der Wissenschaften*, Bd. IV, 1911.
12. PEROLD, A. J. *Centralbl. für Bakt.* Abt. II, Bd. XXIV, 1909.
13. RAYNER, M. C. *Annals of Botany*, Vol. XXIX, No. C. XIII, 1915.
14. WARD, MARSHALL. *Annals of Botany*, Vol. IX, 1895.

(*Received April 25th*, 1917.)

The Journal of Agricultural Science is issued in quarterly parts of about 100 pages with plates and figures, four parts constituting a volume.

Volumes I, II, III, IV, V, VI, VII and VIII now ready. In Four Parts, paper covers, 15*s. net* per volume. Bound in Buckram, 18*s.* 6*d. net* per volume.

Papers for publication may be sent to Professor T. B. Wood, M.A., School of Agriculture, Cambridge, or to the associate Editors. Other communications should be addressed to the University Press, Cambridge.

Contributors will receive twenty-five copies of their papers free. A limited number of additional copies may be obtained at cost price if ordered when final proof is returned.

The subscription price, payable in advance, is 15*s.* per volume (post free); single numbers 5*s.* net. Subscriptions may be sent to any Bookseller, or to Mr C. F. Clay, Manager, Cambridge University Press, Fetter Lane, London, E.C. 4

The Cambridge University Press has appointed the University of Chicago Press agents for the sale of *The Journal of Agricultural Science* in the United States of America and has authorised them to charge the following prices:—Subscription $3·75 net per volume; single copies, $1·25 net.